

## SHORT COMMUNICATION

### Allometry of locomotor organs and sexual size dimorphism in the mygalomorph spider *Grammostola rosea* (Walckenaer, 1837) (Araneae, Theraphosidae)

**Bruno Grossi<sup>1</sup>, Claudio Veloso<sup>1</sup>, Andrés Taucare-Ríos<sup>1,2</sup> and Mauricio Canals<sup>3</sup>:** <sup>1</sup>Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile; <sup>2</sup>Instituto de Ecología y Biodiversidad, Facultad de Ciencias, Universidad de Chile; <sup>3</sup>Departamento de Medicina and Programa de Salud ambiental, Escuela de Salud Pública, Facultad de Medicina, Universidad de Chile. E-mail: mcanals@uchile.cl

**Abstract.** Although sexual size dimorphism is a widely observed phenomenon in nature, the selective forces that led to it are still controversial. Here we study sexual dimorphism in the static allometry of the legs of a large ground spider, *Grammostola rosea* (Walckenaer, 1837). We found that this species has a moderate sexual size dimorphism and males have longer legs relative to body size than females, similar to other ground spiders. We propose that male mate searching behavior may be a relevant factor in the genesis of this phenomenon. The longer extremities in males with respect to mass than in females would lead to an optimization of the costs associated with locomotion, because males have smaller masses and longer legs than the females both in absolute terms and relative to body mass.

**Keywords:** Allometry, sexual dimorphism, spiders, Mygalomorphae

Gender specific differences in locomotor structures have usually been attributed to a more active behavior of one sex, typically males (Gasnier et al. 2002). However, as we discuss below, gender-specific elongation of limbs may not only be associated with locomotion (Framenau 2005).

Ground-living spiders are less sexually dimorphic in size than web-building species, which has been attributed to their differing reproductive and foraging strategies (Prenter et al. 1999). There is evidence for sexual dimorphism in locomotor structures in ground-living spiders (Gasnier et al. 2002; Framenau 2005). Montgomery (1910) reported that males have relatively longer legs than females, suggesting that this is a result of the nomadic behavior of males after attaining sexual maturity. This idea is supported by studies on the locomotor activity of wolf spiders, in which males were the more active sex (Framenau et al. 1996; Framenau 2005), but see Aisenberg et al. (2010) for a counterexample. Morphometric data on leg length in wolf spiders showed comparatively longer legs for males than females only after the final molt, suggesting its significance in reproductive behavior such as searching for mates (Framenau 2005).

Although sexual dimorphism has been reported in mygalomorph spiders (Calderon et al. 1990; Costa & Pérez-Miles 2002; Santos 2007) to our knowledge there are no allometric studies of locomotor organs in these species. As in wolf spiders, adult males of mygalomorph spiders may be expected to show longer legs than females as a consequence of positive allometry. The aim of this study is to compare the allometric relationships between leg length and body mass in both sexes of the spider *Grammostola rosea* (Walckenaer, 1837) (Theraphosidae), a wandering mygalomorph spider in which the male actively searches for females during the reproductive season.

Like other Theraphosidae, *G. rosea* is a large, wandering mygalomorph spider with sexual size dimorphism (Costa & Pérez-Miles 2002). It inhabits mainly arid and semi-arid regions in the lowlands near mountain environments of sclerophyllous forest and Mediterranean scrubland, i.e., habitats characterized by cold, wet winters and hot, dry summers. It is a species of terrestrial habitats; it may be found in areas of low vegetation or in specific areas on slopes or soft ground, where it builds burrows up to 45 cm deep (Canals et al. 2007; Alfaro et al. 2013).

Fifty adults individuals, 24 females ( $16.93 \pm 2.37$  g; mean  $\pm$  SD) and 26 males ( $10.10 \pm 1.19$  g) were captured in Colina, north of Santiago, Chile ( $33^{\circ}11'S$ ,  $70^{\circ}40'W$ ). The individuals were taken to the laboratory and kept in individual terraria ( $24 \times 12 \times 10$  cm) at  $25^{\circ}C$  with a 12h:12h L:D photoperiod and water *ad libitum*. The spiders were fed weekly with five larvae of *Tenebrio molitor* to maintain the body weight at capture, based on estimations of Canals et al. (2012).

Each spider was immobilized via a dorso-ventral compression elastic device applied to the prosoma of the spider. Each leg and the palps were extended manually and fixed with staples in the femur. Then each spider and a reference mark of 50 mm were photographed with a NIKON D70 camera. The image file was analyzed with a morphometric software (ImageJ 1.47b software<sup>®</sup>), measuring the body length and the total length of each leg and palp of the right side considered as the sum of the lengths of the segments, the dorsal area of the prosoma and the dorsal area of the opistosoma.

Data were analyzed with R-software, considering a significance level  $\alpha = 0.05$ . For all variables, normality and homoscedasticity were tested with the Kolmogorov-Smirnov and Levene tests, respectively. Difference in body mass between sexes was analyzed with Student's *t* test for independent samples. Potential regressions ( $y = ax^b$  or equivalently  $\log(y) = \log(a) + b\log(x)$ , with *x* and *y* as the independent and the dependent variables, respectively) were performed for body length, prosoma area, opistosoma area, and leg length (L1–L4) with respect to body mass for each sex. To compare the allometric relationships of the sexes, an ANCOVA was used to test for homogeneity of slopes with body mass as a co-variable. The allometric exponents ( $\beta$ ) were compared with those expected by isometry with Student *t* tests:  $t_{n-2} = (\beta - E(\beta))/SE(\beta)$ , with the expected value  $E(\beta) = 1/3$  for lengths and  $E(\beta) = 2/3$  for areas. As all tests were repeated seven times (one time for each variable), the Bonferroni correction for *P*-value was used.

To study multivariate sex differences, comparisons of L1 to L4 (but standardized (L1<sub>s</sub> to L4<sub>s</sub>) by the cube root of the body mass) were performed with variance analysis for repeated measures (legs). Also, principal components and discriminant analyses were performed to find variables that explain the total variability and the sexual dimorphism, respectively.

Table 1.—Morphological characteristics (Value columns) and allometric relationships among different morphological variables and body size in males and females of the spider *Grammostola rosea*.  $M_b$  is the body mass, L1–L4 are length of legs 1 to 4, BL, PSA and OPA are body length, prosoma area and opisthosoma area, respectively. \*\* indicates a  $P$ -value  $<< 0.001$  for sexual differences.  $F$  is the  $F$ -test with  $H_0: b = 0$ ,  $b$  the regression coefficient, and  $P$  the  $P$ -value of the allometric relationship. Also, the  $P$ -value for homogeneity of slopes between sexes in ANCOVA test is shown ( $P_{sh}$ ), and \* indicates differences with the expected values by isometry (1/3 for lengths and 2/3 for areas) in the student  $t$ -test.

Variable	Males				Females				$P_{sh}$
	Value	$F_{(1,24)}$	$b$	$P$	Value	$F_{(1,22)}$	$b$	$P$	
$M_b$ (g)	$10.10 \pm 1.19^{**}$				$16.93 \pm 2.37$				
BL (cm)	$4.25 \pm 0.22^{**}$	34.82	0.34	$<0.001$	$5.06 \pm 0.30$	30.34	0.33	$<0.001$	0.92
PSA (cm <sup>2</sup> )	$6.13 \pm 0.28^{**}$	36.3	0.61	$<0.001$	$5.82 \pm 0.24$	13.44	0.41*	0.001	0.18
OPA (cm <sup>2</sup> )	$5.80 \pm 0.22^{**}$	18.03	0.83	$<0.001$	$5.39 \pm 0.20$	14.26	0.64	0.001	0.46
L1 (cm)	$5.37 \pm 0.25^{**}$	34.18	0.3	$<0.001$	$5.08 \pm 0.17$	21.31	0.22*	$<0.001$	0.24
L2 (cm)	$6.44 \pm 0.26^{**}$	23.03	0.33	$<0.001$	$6.13 \pm 0.19$	20.3	0.19*	$<0.001$	0.08
L3 (cm)	$2.89 \pm 0.26^{**}$	30.23	0.33	$<0.001$	$3.71 \pm 0.33$	14.25	0.16*	0.001	0.04
L4 (cm)	$2.54 \pm 0.36^{**}$	30.49	0.25	$<0.001$	$4.54 \pm 0.68$	8.47	0.12*	0.008	0.04

A clear sexual size dimorphism was found in all variables and all variables showed positive correlation with body mass in both sexes (Table 1). The allometric exponents were similar between the sexes except for L3 and L4, which showed marginal differences. The relationships of all variables with body mass were isometric in males, while females showed negative allometry in all variables except body length and the area of the opisthosoma.

Sexual differences in body length and prosoma area disappeared when body mass was used as covariate ( $F_{1,47} = 0.003$ ,  $P = 0.96$  and  $F_{1,47} = 0.012$ ,  $P = 0.91$ , respectively). However, opisthosoma area showed differences which were not explained by body mass ( $F_{1,47} = 8.04$ ,  $P = 0.007$ ).

There were differences in the standardized lengths of legs L1<sub>s</sub> to L4<sub>s</sub> ( $F_{3,141} = 861.9$ ,  $P << 0.001$ ) and between sexes ( $F_{1,47} = 15.2$ ,  $P < 0.001$ ); an interaction between leg length and sex was found ( $F_{3,141} = 8.4$ ,  $P < 0.001$ ) (Fig. 1).

Principal components analysis of the standardized variables (by  $M_b^{1/3}$  in the case of lengths and  $M_b^{2/3}$  in the case of areas) showed that the first two components explained 81.4% of the total variance. The first axis was affected positively by L1<sub>s</sub> to L4<sub>s</sub> and prosoma area and negatively by the opisthosoma area, and the second axis was mainly explained by body length. Discriminant analysis showed a complete separation between males and females ( $\Lambda$ -Wilks = 0.024,  $F_{8,40} = 202.98$ ,  $P << 0.001$ ) with 100% correct classification. The discriminant phenetic axis was supported only by differences in L2<sub>s</sub> ( $\Lambda$ -Wilks = 0.027,  $F_{8,40} = 5.84$ ,  $P = 0.02$ ) and L4<sub>s</sub> ( $\Lambda$ -Wilks = 0.028,  $F_{8,40} = 6.89$ ,  $P = 0.01$ ).

Sexual size dimorphism is common in spiders (Moya-Laraño et al. 2002, 2009; Brandt & Andrade 2007). Our results showed a clear sexual size dimorphism in *G. rosea*, though not extreme because the body mass of males was 59.66% that of females, while in some web spiders of the Theridiidae family, males have 1% of the female body mass. This result agrees with those reported in other ground spiders (Gasnier et al. 2002; Framenau 2005), but is lower than those reported for orb web spiders with sexual size dimorphism (Hormiga et al. 2000).

Males of *G. rosea* showed an interesting isometry in all variables, indicating that an increment in body mass is accompanied by a proportional increment of all locomotor body parts. In contrast, the females only showed isometric growth in the opisthosoma area, while all other variables showed a negative allometric growth. This may be a consequence of favoring the development of the reproductive system instead of developing the locomotor system during the ontogeny of females. Although the abdomen size should be associated with increased fitness, this was decoupled with the growth of the legs, indicating that the females do not encounter selective pressures favoring increased

locomotor efficiency. The opisthosoma area of the females was larger than that of males (standardized by body mass) which may be a consequence of the presence of the large reproductive system in females compared to that of males. The large body mass of the females results in greater force on the legs and could explain the shortening of the limbs relative to that of males. Also, the body mass of females could explain the more pronounced negative allometry in legs 3 and 4, because the biomechanics of spider locomotion may be composed by two successive quadrupeds in series, being the second L3-R3-L4-R4 (L and R, left and right) (Biancardi et al. 2011) and these two legs support the torque caused by the weight of the large abdomen of the female.

These results and those of multivariate analysis show a robust sexual size dimorphism in this species; males have smaller size, longer legs and a shorter opisthosoma than do females. Sexual size dimorphism may come about by different combinations of factors and selective pressures: i) by an increase only in the size of the female, for example as a consequence of a correlation between body size and clutch size as postulated by the fecundity hypothesis in other spider species (Head 1995; Prenter et al. 1999); ii) by the reduction of male size only, which is postulated by several hypotheses (Ghiselin 1974; Reiss 1989; Vollrath & Parker 1992; Elgar & Fahey 1996; Moya-Laraño et al. 2002, 2009; Grossi & Canals 2015); iii) by an increase in female size and a decrease in male size; and iv) by an increase or decrease of the size in both sexes but at different rates. Hormiga et al. (1995, 2000) in a phylogenetic analysis with 80 genera of spiders showed that the monomorphic trait in spiders is a primitive condition and that sexual size dimorphism is a derived character which in some cases has been inverted; on more than five occasions in one family (Araneae, Argiopidae).

Sexual differences in locomotor organs in spiders may be favored both by selection on male mate-searching behavior and by natural selection of female movements in relation to foraging and oviposition (Framenau & Hebets 2007). However, sexual differences in locomotor organs may not be related to an advantage in locomotion. For example, elongated legs in males has been attributed to direct male competition for mates in the water strider *Gigantometra gigas* (Tseng & Rowe 1999) and megalopodine beetles (Eberhard & Marin 1996), in male courtship displays in wolf spiders (Kronstedt 1990; Framenau & Hebets 2007) and to reduce the risk of sexual cannibalism in some orb-web spiders because females may either fail to detect very small males, or ignore them as potential prey items. (Elgar et al. 1990).

Sexual dimorphism in locomotor structures seems widespread in ground living spiders (Calderon et al. 1990; Gasnier et al. 2002; Framenau 2005). For example, Gasnier et al. (2002) reported that males had higher area than females because they have longer legs relative to the



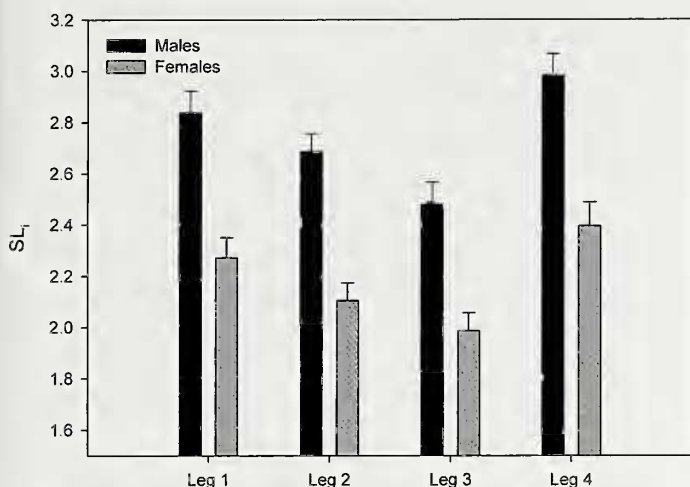


Figure 1.—Standardized length of legs ( $SL_i$ ,  $i = 1-4$ ) of males and females of the spider *Grammostola rosea*.

prosoma length than females in the spiders *Phoneutria* spp. and *Ctenus* spp. (Ctenidae). Framenau (2005) reported that in the wolf spider *Venatrix lapidosa* (McKay, 1974) which has sedentary females, males had comparatively longer legs than females, while in *Artoria* sp. which has vagrant females, there was no gender-specific difference in relative leg length. This suggests that leg length is associated with the locomotor activity of spiders and provides further evidence that limb elongation in males mainly arises due to indirect male competition for mates.

From an energetic point of view, male spiders with longer legs may have increased locomotor efficiency (Ghiselin 1974; Grossi & Canals 2015). Long legs are related to high speed, which may be determinant in opportunities to copulate, especially in the case of Theraphosidae in which walking males may be concentrated in time and space and have a short lifespan compared to that of females (Costa & Pérez-Miles 2002). High speed and low energy expenditure or cost of transport should be favored by natural selection. Pendulum mechanics show the advantages of long legs in spiders and their relationship to high speed, especially in climbing and bridging spiders. Small size is related to low cost of transport (Grossi & Canals 2015). Thus small size compensated by long legs should be the expected morphology for a fast and mobile male spider, agreeing with our results with *G. rosea*.

## ACKNOWLEDGMENTS

We thank Lafayette Eaton for helpful comments on the manuscript. The present study was funded by a FONDECYT 1110058 grant to M.C.

## LITERATURE CITED

Aisenberg, A., F.G. Costa, M. Gonzalez, R. Postiglioni & F. Pérez-Miles. 2010. Sexual dimorphism in chelicerae, forelegs and palpal traits in two burrowing wolf spiders (Araneae: Lycosidae) with sex-role reversal. *Journal of Natural History* 44:19–20.

Alfaro, C., D.P. Figueroa, H. Torres-Contreras, F. Venegas, L. Canals & M. Canals. 2013. Effect of thermal acclimation on preferred temperatures in two mygalomorph spiders inhabiting contrasting habitats. *Physiological Entomology* 38:20–25.

Biancardi, C.M., C.G. Fabrica, P. Polero, J.F. Loss & A.E. Minetti. 2011. Biomechanics of octopodal locomotion: kinematic and kinetic analysis of the spider *Grammostola mollicona*. *Journal of Experimental Biology* 214:3433–3442.

Brandt, Y. & M.C.B. Andrade. 2007. Testing the gravity hypothesis of sexual size dimorphism: are small males faster climbers? *Functional Ecology* 21:379–385.

Calderon, R., M. Garrido & C. Pinto. 1990. Etapas del crecimiento de *Acanthognathus franckii* Karsch, 1880 (Araneae: Nemesidae). *Revista Chilena de Entomología* 18:19–24.

Canals, L., D.P. Figueroa, H. Torres-Contreras, C. Veloso & M. Canals. 2012. Are mealworm (*Tenebrio molitor*) diets in concordance with energetic requirements of small mygalomorph spiders (*Paraphysa* sp.)? *Journal of Exotic Pet Medicine* 21:203–206.

Canals, M., M.J. Salazar, C. Durán, D. Figueroa & C. Veloso. 2007. Respiratory refinements in the mygalomorph spider *Grammostola rosea* Walckenaer 1837 (Araneae, Theraphosidae). *Journal of Arachnology* 35:481–486.

Costa, M. & F. Pérez-Miles. 2002. Reproductive biology of Uruguayan theraphosids (Araneae, Mygalomorphae). *Journal of Arachnology* 30:571–587.

Eberhard, W.G. & M.C. Marin. 1996. Sexual behavior and the enlarged hind legs of male *Megalopus armatus* (Coleoptera, Chrysomelidae). *Journal of the Kansas Entomological Society* 69:1–8.

Elgar, M.A. & B.F. Fahey. 1996. Sexual cannibalism, competition, and size dimorphism in the orb-weaving spider *Nephila plumipes* Latreille (Araneae: Araneoidea). *Behavioral Ecology* 7:195–198.

Elgar, M.A., N. Ghaffar & A.F. Read. 1990. Sexual dimorphism in leg length among orb-weaving spiders: a possible role for sexual cannibalism. *Journal of Zoology, London* 222:455–470.

Framenau, V.W. 2005. Gender specific differences in activity and home range reflect morphological dimorphism in wolf spiders (Araneae, Lycosidae). *Journal of Arachnology* 33:334–346.

Framenau, V.W. & E.A. Hebets. 2007. A review of leg ornamentation in male wolf spiders, with the description of a new species from Australia, *Artoria schizocoides* (Araneae, Lycosidae). *Journal of Arachnology* 35:89–101.

Framenau, V., M. Reich & H. Plachter. 1996. Zum Wanderverhalten und zur Nahrungsökologie von *Arctosa cinerea* (Fabricius, 1777) (Araneae: Lycosidae) in einer alpinen Wildflusslandschaft. *Verhandlungen der Gesellschaft für Ökologie* 26:369–376.

Gasnier, T.R., C.S. de Azevedo, M.P. Torres-Sanchez & H. Hofer. 2002. Adult size of eight hunting spider species in central Amazonia: temporal variations and sexual dimorphism. *Journal of Arachnology* 30:146–154.

Ghiselin, M.T. 1974. *The Economy of Nature and the Evolution of Sex*. University of California Press, Berkeley.

Grossi, B. & M. Canals. 2015. Energetics, scaling and sexual size dimorphism of spiders. *Acta Biotheoretica* 63:71–81.

Head, G. 1995. Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (class Araneae). *Evolution* 49:776–781.

Hormiga, G., W.G. Eberhard & J.A. Coddington. 1995. Web-construction behavior in Australian Phonognatha and the phylogeny of nephiline and tetragnathid spiders (Araneae: Tetragnathidae). *Australian Journal of Zoology* 43:313–364.

Hormiga, G., N. Scharff Coddington & J.A. Coddington. 2000. The phylogenetic basis of sexual size dimorphism in orb-weaving spiders (Araneae: Orbiculariae). *Systematic Biology* 49:435–462.

Kronstedt, T. 1990. Separation of two species standing as *Alopecosa aculeata* (Clerck) by morphological, behavioural and ecological characters, with remarks on related species in the *pulverulenta* group. *Zoologica Scripta* 19:203–225.

Montgomery, T.H. 1910. The significance of the courtship and secondary sexual characters of araneids. *American Naturalist* 44:151–177.

Moya-Laraño, J., J. Halaj & D.H. Wise. 2002. Climbing to reach females: Romeo should be small. *Evolution* 56:420–425.

- Moya-Laraño, J., D. Vinkovic, C.M. Allard & M.W. Foellmer. 2009. Optimal climbing speed explains the evolution of extreme sexual size dimorphism in spiders. *Journal of Evolutionary Biology* 22:954–963.
- Prenter, J., R.W. Elwood & W.I. Montgomery. 1999. Sexual size dimorphism and reproductive investment by female spiders: a comparative analysis. *Evolution* 53:1987–1994.
- Reiss, M. J. 1989. *The Allometry of Growth and Reproduction*. Cambridge University Press, Cambridge.
- Santos, A.J. 2007. Evolucao do dimorfismo sexual de tamanho em aranhas. Pp. 137–165. *In* *Ecologia e Comportamento de Aranhas*. (M.O. Gonzaga, A.J. Santos, H.F. Japyassú, eds.). Editora Inter-ciência, Rio de Janeiro.
- Tseng, M. & L. Rowe. 1999. Sexual dimorphism and allometry in the giant water strider *Gigantometra gigas*. *Canadian Journal of Zoology* 77:923–929.
- Vollrath, F. & G.A. Parker. 1992. Sexual dimorphism and distorted sex ratios in spiders. *Nature* 360:156–159.

*Manuscript received 13 July 2015, revised 6 January 2016.*